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Plasticity of the Muscle Proteome to Exercise at Altitude

Martin Flueck

Abstract

Flueck, Martin. Plasticity of the muscle proteome to exercise at altitude. *High Alt. Med. Biol.* 10: 183–193, 2009.—The ascent of humans to the summits of the highest peaks on Earth initiated a spurt of explorations into the physiological consequences of physical activity at altitude. The past three decades have demonstrated that the resetting of respiratory and cardiovascular control with chronic exposure to altitudes above 4000 m is accompanied by important structural–functional adjustments of skeletal muscle. The fully altitude-adapted phenotype preserves energy charge at reduced aerobic capacity through the promotion of anaerobic substrate flux and tighter metabolic control, often at the expense of muscle mass. In seeming contrast, intense physical activity at moderate hypoxia (2500 to 4000 m) modifies this response in both low and high altitude natives through metabolic compensation by elevating local aerobic capacity and possibly preventing muscle fiber atrophy. The combined use of classical morphometry and contemporary proteomic technology provides a highly resolved picture of the temporal control of hypoxia-induced muscular adaptations. The muscle proteome signature identifies mitochondrial autophagy and protein degradation as prime adaptive mechanisms to passive altitude exposure and ascent to extreme altitude. Protein measures also explain the lactate paradox by a sparing of glycolytic enzymes from general muscle wasting. Enhanced mitochondrial and angiogenic protein expression in human muscle with exercise up to 4000 m is related to the reduction in intramuscular oxygen content below 1% (8 torr), when the master regulator of hypoxia-dependent gene expression, HIF-1α, is stabilized. Accordingly, it is proposed here that the catabolic consequences of chronic hypoxia exposure reflect the insufficient activation of hypoxia-sensitive signaling and the suppression of energy-consuming protein translation.

Key Words: hypoxia; oxygen; cellular respiration; buffer; energy charge; HIF-1

Introduction

ATP production by the oxidative combustion of organic substrates in mitochondria is the single most important determinant of maximal metabolic rate (Arthur et al., 1992; Hochachka, 1998; Bickler and Buck, 2007; Flück et al., 2007). Longer-lasting reductions in ATP synthesis due to a lowering of oxygen concentration have critical consequences for cell function (Bickler and Buck, 2007). In these situations of reduced cellular respiration, the energetically less efficient anaerobic limb of glycolysis is increased, and energy-intensive cellular processes are shut down to preserve energy charge, that is, $ATP + ½ADP/(ATP + ADP + AMP)$* (Atkinson and Chapman, 1979; Spriet, 1992), and to prevent harmful consequences due to misbalanced oxidative processes (Askew, 2002; Bickler and Buck, 2007).

The central control of energy metabolism by oxygen is highlighted by the drop in maximal aerobic power output during muscle work in hypoxia (West,1990; Green et al., 1999; Geiser et al., 2001; Vogt et al., 2001; Beidleman et al., 2003). This reduction is proportional to oxygen tension in working muscle and amounts to approximately 0.5%/100 m of elevation in altitude (Fulco et al., 1998). This has dire consequences for exercise performance and work capacity at altitudes. At heights 8500 m above sea level, maximal energy expenditure is lowered to critical levels only slightly above basal metabolic rate (Howald and Hoppeler, 2003). Physical work at these extremes has important physiological implications and is impossible without acclimatization.

The influence of high altitude on respiratory and cardiovascular function was documented nearly a century ago in studies at the top of Pikes Peak (4300 m) in Colorado (Reeves

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*ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate.

et al., 1992). The human explorations to the highest peaks on Earth after World War II extended the knowledge on acclimatization to extreme altitudes. The subsequent introduction of the muscle biopsy technique in the 1960s (Bergstrom et al., 1967) provided experimental biologists with a tool to probe alterations in skeletal muscle, which was suspected at the time to show a certain degree of plasticity (Hoppeler et al., 2003; Howald and Hoppeler, 2003). The early cellular and biochemical investigations pointed out that a suite of local muscle reactions (Howald and Hoppeler, 2003) accompanies

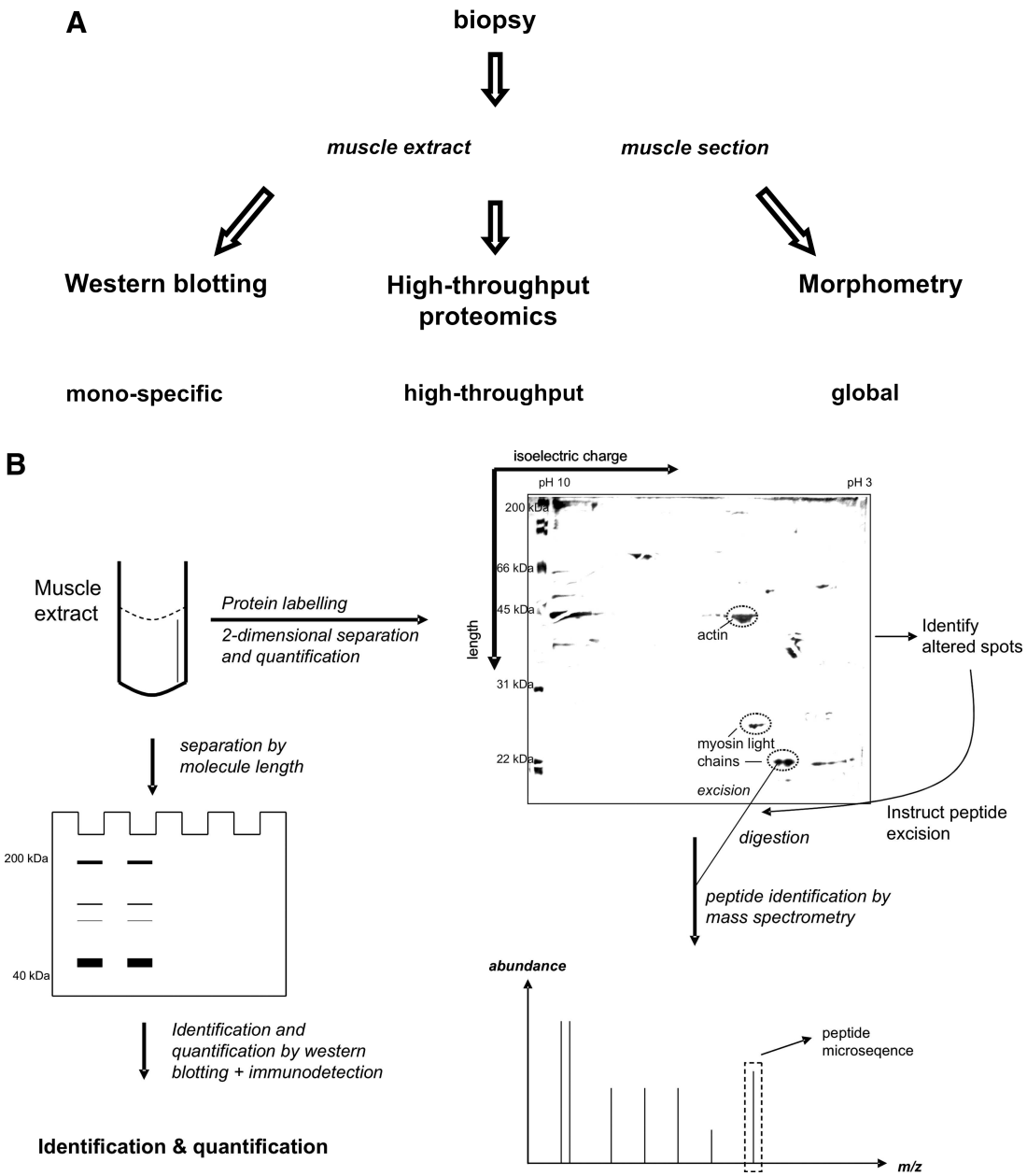

FIG. 1. (**A**) Overview and (**B**) summary of the main steps of protein quantification by monospecific and high-throughput biochemical techniques and (**C**) a global microscopic approach. (B, left) Muscle protein extract is denatured and proteins separated by molecule length with sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Proteins are transferred to a membrane by western blotting, and selected proteins are immunodetected with monospecific antibodies. (B, right) Extracted proteins of one sample are labeled and separated using two-dimensional gel electrophoresis, first as native proteins for isoelectric charge against a pH gradient, then after denaturation in SDS for molecule length (in kilodaltons, kDa). A representative example from rat tibialis anterior muscle is shown. Signal intensity of the individual protein spots is normalized to total label and compared between experiments. Significantly altered proteins are identified by peptide microsequencing of excised protein spots (circled) with peptide digestion and mass spectrometry (below). (C) Muscle sections are visualized with light microscopy and electron microscopy, and the volumetric contribution of (sub)cellular structures is quantified with morphometry. This involves the determination of areas and numbers of cellular elements by unbiased counting along intersections of a test system (grid) in randomly sampled visual fields of the muscle section. The volumetrically most abundant structures are numbered 0, muscle fiber; 1, myofibrils; 2; mitochondria; 3, capillaries; 4, sarcoplasmic reticulum and t-tubuli; 5, nuclei. Data from all three basic approaches are included in this review.

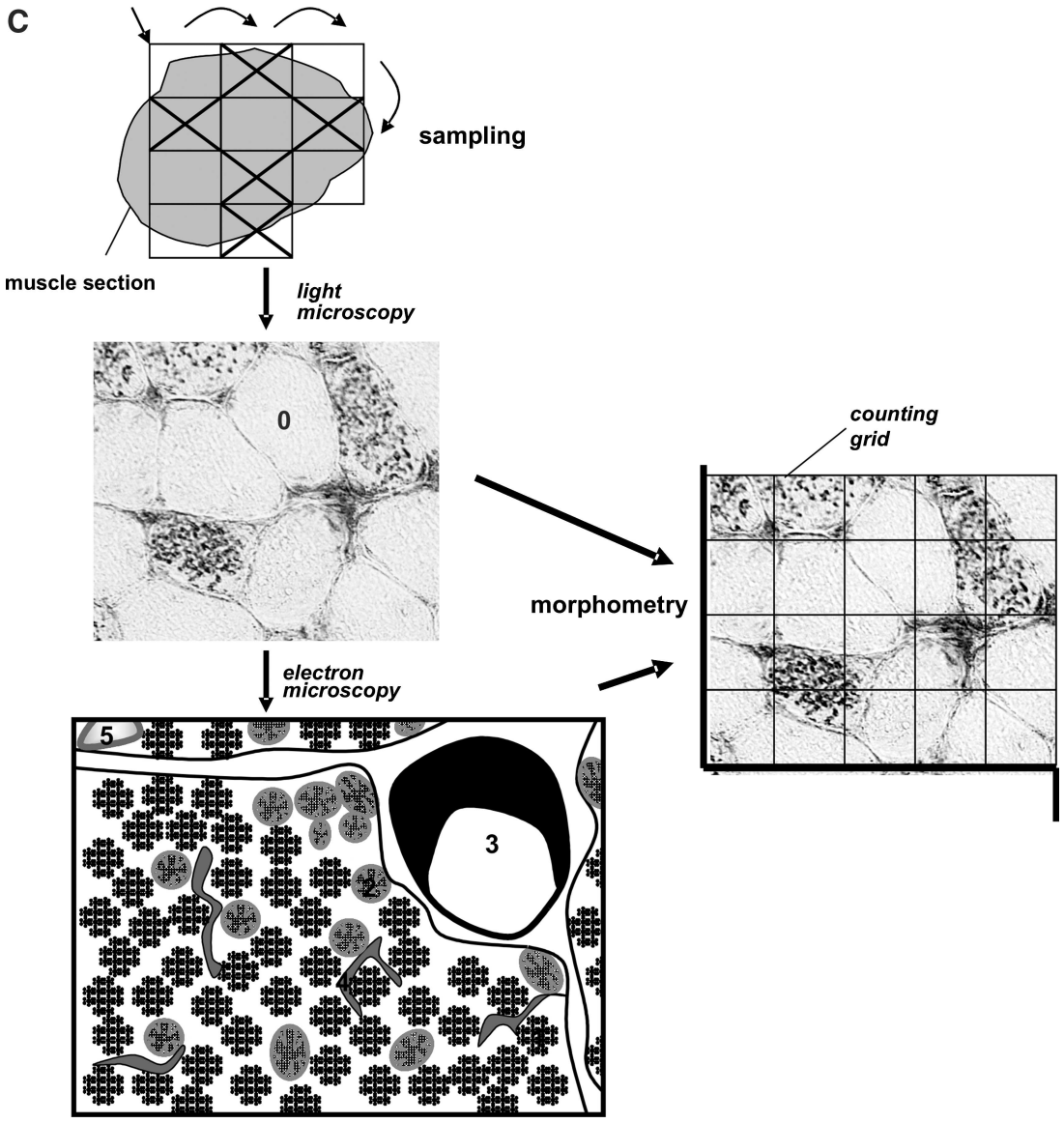

FIG. 1. Continued.

the cardiopulmonary adjustments to high altitude exposure in the body's attempt to reestablish homeostasis (Bailey and Davies, 1997). The consensus view is that muscle adjustments to low levels of hypoxia shift metabolic enzyme activity toward greater aerobic poise; extreme hypoxia shifts metabolism toward greater anaerobic potential with higher perfusion (Clanton and Klawitter, 2001; Howald and Hoppeler, 2003). Exercise importantly modifies this response and improves aerobic performance at altitude and possibly at sea level.

Recent molecular investigations point to an important contribution of gene-regulated muscle protein turnover to the adaptations of human metabolic performance with exercise at high altitude. This review will address the emerging molecular mechanisms that underlie hypoxia-modulated muscle remodeling by protein expression. A focus will be put on recently developed high-throughput approaches that allow identifying quantitative alterations of multiple protein species. Emphasis is put on the integration of molecular alterations with established structural–functional adjustments to exercise in a hypoxic environment in regard to the varied role of muscle oxygen tension for sea-level and high altitude natives.

Due to space restrictions, not all relevant literature could be integrated into this review.

The Muscle Proteome

Protein measures are the ultimate means to resolve the fine molecular adjustments that become manifest as structural adaptations of protein assemblies (organelles), cells, and organs. Classically, this analysis is achieved in a wet lab using immunological techniques for selected, single protein species, for example, western blotting, enzyme-linked immunosorbent assay (ELISA), or immunofluorescence. The recent combination of classic protein separation by two-dimensional gel electrophoresis and microsequencing by mass spectrometry has established powerful technology to identify an important segment of all expressed proteins (the proteome) in parallel (Fig. 1; Hojlund et al., 2008).

In the context of the young age of proteomic technology, it is worthwhile to consider that not all of the possible protein products encoded by the 30,000 genes of the human genome are sufficiently abundant to be identified directly by mass-spectrometry-based microsequencing (Hojlund et al., 2008). Low sensitivity and the adoption of a conservative cutoff are the main limitations of current approaches. Therefore, the current description of the human muscle proteome essentially represents protein species that make up the bulk of the muscle's contractile and metabolic machinery and the associated endothelial cell type (Schwerzmann et al., 1989; Gelfi et al., 2006; Hojlund et al., 2008). Adjustments in lower-abundant proteins are not typically quantified in routine proteome approaches that identify candidate species based on sizable intensity changes of protein spots in two-dimensional maps (Hojlund et al., 2008). For lower-expressed protein species, the specific, but indirect, detection with immunological techniques is still the method of choice (Viganò et al., 2008). These considerations possibly explain why astonishingly few proteomic differences are evident between phenotypically distinct muscles. Thus, currently, quantitative microscopy (morphometry) of cell structures and organelles, which reflect protein assemblies, offers a valuable alternative for estimating the global contribution of protein changes (Schwerzmann et al., 1989). At this juncture it is important to bear in mind that proteome measures provide only concentration-related values. With changes in muscle volume, it is therefore important to view the identified relative changes in muscle protein in the perspective of absolute measures of muscle mass.

Shifts in the muscle proteome with altitude exposure

The first study into the effect of altitude on muscle protein by Reynafarje (1962) reported an elevated content of the oxygen-binding protein myoglobin (+16%) and cytochrome c reductase activity (+78%) in biopsies of the hip flexor muscle sartorius of permanent residents of Peru (3600 m above sea level) in comparison to lowlanders. This observation and the later finding on elevated mitochondrial volume densities in slow and fast muscle fibers in patients with unilateral intermittent claudication shaped the belief that hypoxia alone is the stimulus for aerobic metabolism (Angquist and Sjostrom, 1980; Terrados et al., 1990).

This view was questioned by a series of studies pointing out that residency at altitude shifts muscle to a more glycolytic type, with tighter coupling of energetic processes and "elevated buffer capacity," which reduces lactate efflux during muscle work, the lactate paradox (Brooks et al., 1991; Reeves et al., 1992; Desplanches et al., 1996; Kayser et al., 1996; Allen et al., 1997; Clanton and Klawitter, 2001) These alterations in high altitude natives are accompanied by atrophy in muscle fibers. It is understood that this shrinkage of muscle fibers optimizes capillary diffusion distances in the altitude-acclimatized muscle phenotype (Howald and Hoppeler, 2003). Notably, the changes in fiber size and capillarity with altitude exposure above 4000 m are qualitatively similar between lowlanders and altitude-adapted populations (Table 1). These commonalities in local adjustments suggest that fiber atrophy serves a same purpose to optimize metabolic supply to working muscle. However, the mechanisms underlying the acute muscle alterations of lowlanders seemingly differ from those maintaining the muscle steady state of high altitude natives, because the acquired metabolic features in the former population do deacclimate (Hochachka et al., 1991).

Muscle activity modifies the effects of high altitude on mitochondrial biogenesis and fiber growth

Contractile activity is a potent stimulator of mitochondrial biogenesis in skeletal muscle (Hood, 2001). The reinterpretation of mitochondrial protein and ultrastructure in leg muscles of high altitude populations indicates that muscle activity importantly interacts with the stimulus of altitude by promoting aerobic metabolism (Desplanches et al., 1996; Kayser et al., 1996; Hoppeler et al., 2003).

The role of muscle activity for the maintenance of aerobic capacity is highlighted by recent proteome studies on the early altitude acclimatization of lowlanders. The report of Gelfi and colleagues (2008) characterized the muscle protein adjustments after 1 week of rest in the Capanna Regina Margherita in the Monte Rosa Massif of the Swiss-Italian Alps at 4559 m (Viganò et al., 2008). The proteome measures of subjects staying quasi-inactive for 5 days after motorized ascent showed a mixed down- and upregulation of 89 and 33 proteins, respectively, in vastus lateralis muscle. Mass spectrometry identified the extensive downregulation of the key factors of mitochondrial processes, redox regulation, and fiber structure concomitantly with elevated tagging of proteins for proteosomal degradation (Table 1). These data compare to the 3% net loss of muscle mass after 8 days of exposure to similar altitudes (i.e., 4000 m) in nonactive subjects (Kayser, 1994). The new data possibly reflect the earliest sign of muscle fiber wasting with low exercise activity and chronic exposure to moderate heights, similar to what has been seen for cytosolic enzymes at extreme altitudes (Green, 1992). Elevations of red blood cell count and the fractional synthesis rates of the plasma proteins albumin and fibrinogen (Table 1; (Imoberdorf et al., 2001; Robach et al., 2007) illustrate that the vascular compartment improves physiological parameters of altitude-exposed subjects under sedentary conditions at the expense of muscle proteins. The catabolic reactions in muscles of the inactive subjects at altitude are contrasted to the absence of alterations in the abundance of glycolytic factors (Viganò et al., 2008). This implies that the lactate paradox is partially explained by the sparing of glycolytic factors from the general muscle protein degradation at altitude.

The important contribution of muscle activity to proteome adjustments with altitude exposure is supported by the elevated muscle levels of key proteins of aerobic metabolism after the combined impact of exercise in hypoxia (Table 1). Measures on subjects climbing to the heights of the Capanna Regina Margherita indicate the active contribution of protein synthesis to muscle remodeling with exercise at altitude (Table 1; Imoberdorf et al., 2001). Subsequent observations support the tenet that physical activity during exposure to hypoxia modifies the allocation of adjustments; with endurance exercise the contribution of the muscle is pronounced.

Arguably, the investigation of Terrados and colleagues (1990) was the first to appreciate the superior adjustments of the aerobic pathway in muscle when endurance exercise is combined with hypoxia. The one-leg bicycle exercise study demonstrated higher gains in the oxidative marker enzyme citrate synthase (29% vs. 12%), myoglobin (8% vs. −6%), and a gatekeeper of glycolysis, phosphofructokinase (8% vs. 1%) in the muscle that exercised at a simulated altitude of 2300 m versus the contralateral leg that trained at sea level (Terrados et al., 1990). The positive consequence of a living-low, training-high

Table 1. Synopsis of the Literature on Protein Adaptations in Muscle Fibers and Vascular Bed to Exercise at Altitude

Chronic real hypoxia at 3,600–6,000 m								*Simulated intermittent hypoxia at 10–12% oxygen (3,600–6,000 m)*					
one-two weeks		*one month*		*three months*		*generations*		*weeks*	*1 day*	*3–8 weeks*			
inactive	*+exercise*	*inactive*	*+climbing*	*inactive*	*+climbing*	*inactive*	*+exercise*	*No exercise*	*Exercise 30 min 50% VO_2max*	*Exercise 30 min at 65% PPO, 5 times/week, for 6 weeks*	*Proteins assessed*	*Biological process*	*Cell compartment*
↓ −900% (albumin)	nd	nd	nd	nd	↑ +40–300% (↓ −35%Tes)	nd	↑ +10%	↑ +∼40%	↑ +25%	nd	Albumin, EPO, GH, IGF-I, IGF-BP3, PL, Tes	Endocrine regulation	vasculature
nd	nd	nd	nd	nd	↑ +200%	–	↑ +19%–200%	nd	nd	↑ +∼20%	VEGF, capillary	Capillary perfusion	Vascular bed
nd	↑ +35%	nd	nd	nd	nd	nd	nd	nd	nd	nd	Albumin, Fibrinogen	Synthesis serum	vasculature
–	nd	nd	↑ +13%	nd	–	↑ ↓ ±50%	↑ +28%	nd	nd	↑	ENO, LDH, GAPDH, PGM, PFK	glycolysis	muscle
↓ −50%	nd	nd	nd	nd	nd	nd	nd	nd	nd	↑ +25%	AAT	Amino acid combustion	muscle
↓ −50%	nd	nd	nd	nd	↓	↑ +300% ↓ −50%	nd	nd	nd	↓ −40%	CAT, ECH, HADH	Beta oxidation	muscle
↓ −50%	nd	nd	nd	nd	↓ −40%	↓ −50%	↑ +45%	nd	nd	↑ +40–70%	ACO, CS, MDH, ODH	Citrate cycle	muscle
↓ −50%	nd	nd	nd	nd	↓ −30%	↑ +10%	↑ +78%	nd	nd	nd	ATPase, COX, CYCR, ETF, IMMT, NUGM, QCR	Oxidative phosphorylation	muscle
↓ −35%	nd	nd	nd	nd	nd	↑ +120%	↑ +16%	nd	nd	↑ +10%	mb	Oxygen transport	muscle
↓ −50%	↑ +6%	nd	nd	nd	nd	nd	nd	nd	nd	nd	CAIII	Buffer capacity	muscle
↓ ↑	nd	nd	↑	nd	nd	nd	nd	nd	nd	↑ +50%	CK, NMET	Energy transfer	muscle
↓ −50%	nd	nd	nd	nd	nd	↑	nd	↓ −30%	nd	nd	GST-P1, GST-o, PRX2, PRX6, glutathione	detoxification	muscle
nd	nd	nd	–	nd	↓	↓ −13%	nd	nd	nd	↑ ↓	sarcomere	fiber area	muscle
nd	nd	nd	↓ −14%)	nd	nd	nd	nd	nd	nd	↓ −14%	NaATPase, SERCA1	excitation	muscle
–	↑	nd	nd	nd	nd	nd	nd	nd	nd	nd		Protein synthesis	muscle
(Imoberdorf et al., 2001; Kayser, 1994; Robach et al., 2007; Viganò et al., 2008)	(Imoberdorf et al., 2001; Mizuno et al., 1990)	(Howald et al., 1990; Kayser, 1994)	(Green et al., 2000a; Green et al., 2000b; Green et al., 2000c)		(Benso et al., 2007; Green et al., 2000c; Howald et al., 1990; Oelz et al., 1986; Patitucci et al., 2008; Richalet et al., 1994)	Gelfi et al., 2004; Hoppeler et al., 2003; Kayser et al., 1996)	(Desplanches et al., 1996; Patitucci et al., 2008; Reynafarje, 1962; Rosser and Hochachka, 1993; Schmidt et al., 2006)	(Howald et al., 1990; Jefferson et al., 2004; Levine and Stray-Gundersen, 2001)	(Mackenzie et al., 2008)	(Desplanches et al., 1993; Geiser et al., 2001; Green et al., 1999; Melissa et al., 1997; Ponsot et al., 2006; Roels et al., 1990; Vogt et al., 2001)		reference	

Data stem from from single, high-throughput and global protein measures. Selected activity measures are included as well. Underlined values stem from global estimates. Double underlined values from activity measures. ↑ up; ↓ down; −, unaltered; nd, not determined. Abbreviations: ACO, Aconitate hydratase; AAT, Aspartate amino transferase; CAT, carnitine-O-acetyltransferase; CK, creatine kinase; CS, citrate synthase; COX, cytochrome c oxidase; CYCR, cytochrome c reductase; ECH, Δ2-enoyl-CoA-hydratase; ENO, enolase; EPO, erythropoietin; ETF, Electron transfer flavoprotein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GST-o, glutathione-S-transferase omega 1; GST-P1, glutathione-S-transferase p; GH, growth hormone; HADH, hydroxyacyl-CoA dehydrogenase activities; IGF-I, Insulin-like growth factor I, IGF-BP3, Insulin growth factor I binding protein3; LDH, lactate dehydrogenase; Mb, myoglobin; MDH, malate dehydrogenase; IMMT, mitofilin; NUGM, NADH-ubiquinone oxidoreductase; NMET, N-Methyl transferase; ODH, 2-oxoglutarate dehydrogenase; PGM, phosphoglycerate mutase; PFK, phospho fructokinase; PL, prolactin; PRX, Peroxiredoxin; SERCA1, sarco endoplasmic reticulum calcium-ATPase isoform 1; Tes, testosterone; QCR, ubiquinol-cytochrome c reductase; VEGF, vascular endothelial growth factor. PPO, peak power output; VO2max, maximal oxygen uptake.

protocol was validated in several investigations (Green et al., 1999; Geiser et al., 2001; Vogt et al., 2001).

These results lead to reconsiderations of incorporating exercise sessions under a moderate hypoxic stimulus close to 4000 m into macrocycles of exercise training (Bailey and Davies, 1997; Green et al., 1999; Levine and Stray-Gundersen, 2001; Ponsot et al., 2006; Zoll et al., 2006; Roels et al., 2007; Hoppeler and Vogt, 2008). Morphometric estimation of muscle ultrastucture after 6 weeks of repeated exercise under hypoxia demonstrated the translation of individually altered proteins to a global increase in mitochondrial and capillary structures (Table 1). Reduced levels of NaATPase and the sarcoplasmic Ca-ATPase isoform of fast twitch skeletal muscle isoform imply that the muscular adjustments to living-low, training-high include the modified regulation of energy-consuming ion gradients (Green et al., 1999).

A recent trilogy characterizing a related interval type of endurance training in hypoxia versus normoxia counterparts indicated that the metabolic adjustments in endurance athletes comprise a shift in substrate specificity to amino over fatty acids (Roels et al., 2007). Concomitantly, the coupling of mitochondrial ATP production to the recharging of creatine kinase is improved (Ponsot et al., 2006; Zoll et al., 2006) in line with a drive to higher creatine biosynthesis after acclimatization to altitude (Viganò et al., 2008).

Elevations of muscle fiber mass with endurance training in hypoxia also suggest an anabolic effect of hypoxia (Desplanches et al., 1993; Vogt et al., 2001). This increase in muscle fiber mass is also significant with walk training under restricted venous blood flow, known as Kaatsu training (Abe et al., 2006). In contrast, resistance-type exercise in hypoxia equivalent to 4500 m does not induce superior hypertrophy versus matched normoxic exercise, but molecular effects were evident (Masuda et al., 1999; Friedmann et al., 2003).

Zones of Adaptation to Exercise in Hypoxia

Training studies show comparable relative increases in reference enzymes and global estimates of the aerobic pathway in high altitude natives of La Paz, Bolivia (3600 m), and lowlanders (560 m) to 3 to 6 weeks of bicycle endurance training in hypoxia equivalent to 3600 m (Table 1; Desplanches et al., 1996; Vogt et al., 2001). This implies that the basic pathway orchestrating protein upregulation of aerobic metabolism with exercise at altitude is conserved between low- and highlanders.

This effect on mitochondrial volume and fiber size with strenuous exercise is not seen in lowlanders chronically exposed to heights of 3600 to 6000 m (Table 1). Various perturbations at altitude, including starvation due to malnutrition and iron-sparing mechanisms, possibly underlie this detrimental response (Kayser, 1994; Clanton and Klawitter, 2001; Robach et al., 2007). Mitochondria play a central role in all these processes due to their active implication in energy production and the regulation of cell death. This is supported by the selective accumulation of the mitochondrial breakdown product lipofuscin concomitantly with reduced mitochondrial volume density in Caucasian mountaineers after altitude exposure (Martinelli et al., 1990; Gelfi et al., 2004). These alterations resemble mitochondrial autophagy, which removes damaged mitochondria and serves to control muscle fiber atrophy that originates from local fiber death induced by cytochome c release from injured mitochondria (Decker and Wildenthal, 1980; Terman et al., 2004; Gustafsson and Gottlieb, 2008). Mitochondrial damage is suggested by the accumulation of reactive species (radicals) in the muscle's attempt to preserve ATP levels despite critically low oxygen at altitude (Askew, 2002; Terman et al., 2004; Bailey et al., 2008). This implies that the loss of mitochondria and fiber mass reflects the transition to a new metabolic state that overcomes limitations in cellular respiration at altitude by improved diffusion distances between capillary and muscle fiber.

At extreme altitudes above 5000 m, the massive toxic impact of hypoxia comes into full play when muscle protein in lowlanders is strongly reduced despite strenuous muscle work (Howald et al., 1990). Under these circumstances, catabolic reactions prevail despite transient elevations of endocrine and angiogenic factors in serum (Banfi et al., 1994; Benso et al., 2007; Patitucci et al., 2008).

Local Muscle Hypoxia Promotes Oxidative Pathways by HIF-1

Molecular physiological considerations suggest that the drop in muscle oxygen tension due to intense contractile work could be the driving force for the tuning of muscle metabolism. This is thought to be mediated through the activation of mitochondrial biogenesis and angiogenesis by hypoxia-sensitive gene expression (Dapp et al., 2006; Semenza, 2007). Several signaling pathways are implicated in the hypoxia-dependent control of gene expression through the regulation of nuclear transcription factors (Cummins and Taylor, 2005). In skeletal muscle, hypoxia-sensitive signal activation of transcription may occur in the myocellular compartment as a direct consequence of a drop in tissue oxygenation, may result from perturbations in reactive oxygen species or energy status in the myocellular compartment (Breen et al., 2008), or may reflect the response of endothelial cells to the mechanical stress of elevated blood flow in hypoxia (Davies, 1995).

The involvement of signaling to muscle remodeling with lowered oxygenation is well illustrated by the modulation of the master regulator of hypoxia-inducible factor gene expression, HIF-1, with muscle work. This dimeric transcription factor drives the expression of batteries of genes involved in capillary growth, glycolysis, and mitochondrial metabolism in skeletal muscle with hypoxia exposure (Dapp et al., 2006; Semenza, 2007). Hypoxia-dependent regulation by HIF-1 is turned off in well- oxygenated conditions by the degradation of its α-subunit, HIF-1α. With a fall to a critical level of hypoxia, HIF-1α is stabilized, promoting the formation of active HIF-1α,β dimer and inducing activation of downstream gene expression by binding to gene promoters (Stroka et al., 2001).

In culture, the stabilization of HIF-1α is instantaneous below 0.5% to 2% of ambient oxygen (Jewell et al., 2001). This level corresponds to the measured drop of intramuscular oxygen pressure after 20 sec of intense exercise to 8 torr (Fig. 2; Richardson et al., 1999). Correspondingly, stabilization of HIF-1α has been detected after intense endurance exercise in the recruited the knee extensor muscle vastus lateralis (Ameln et al., 2005). These observations imply that HIF-1 activation in muscle results from the sudden fall in resting levels of intramuscular oxygen with exercise-induced oxygen combustion in mitochondria (Richardson et al., 2001; Hoppeler et al., 2003). The relationship between HIF-1 activation and the severity of intramuscular hypoxia is not known, but it is to be

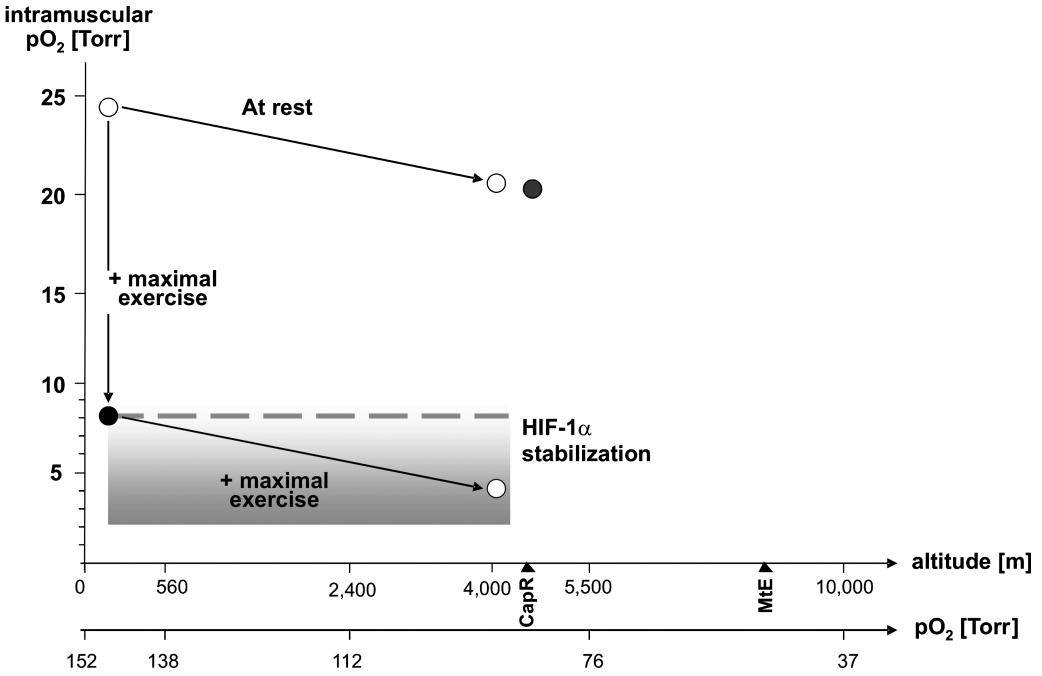

FIG. 2. Composite line graph visualizing the drop in intramuscular pO_2 with exposure at a simulated altitude of 4000 m ($FIO_2 = 0.12$) at rest and in combination with strenuous exercise in relation to HIF-1α stabilization. The heights of Mount Everest (8848 m; MtE) and Capanna Regina (4559 m; CapR) are indicated. Filled circles indicate conditions where HIF-1α protein levels were reported to increase (black filling) or stay unchanged (gray filling). Included data stem from published human and animal experimentation (Richardson et al., 1995; Richardson et al., 2001; Gelfi et al., 2004; Dapp et al., 2006; Richardson et al., 2006; Viganò et al., 2008). A threshold of local oxygen tension at 8 torr is suggested to allow stabilization of HIF-1α in muscle.

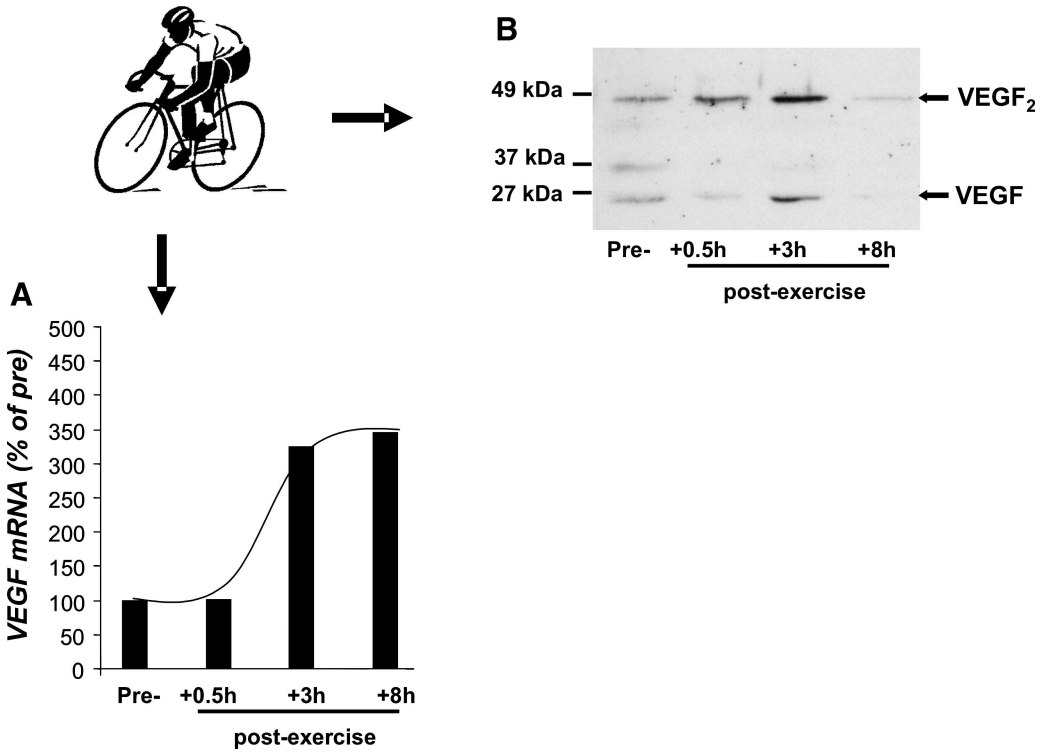

FIG. 3. Acute response of (**A**) RNA and (**B**) protein expression of vascular endothelial growth factor (VEGF) in human vastus lateralis muscle during recovery from a 30-min bout of bicycle exercise in normoxia. Exercise intensity was set to 65% of aerobic power output at 560 m above sea level. Data from one subject are shown. VEGF protein is detected as a monomer (24 kDa) and dimer ($VEGF_2$, 48 kDa). Experiments were conducted in collaboration with Hans Hoppeler and Christoph Daepp (as described in Schmutz et al., 2006).

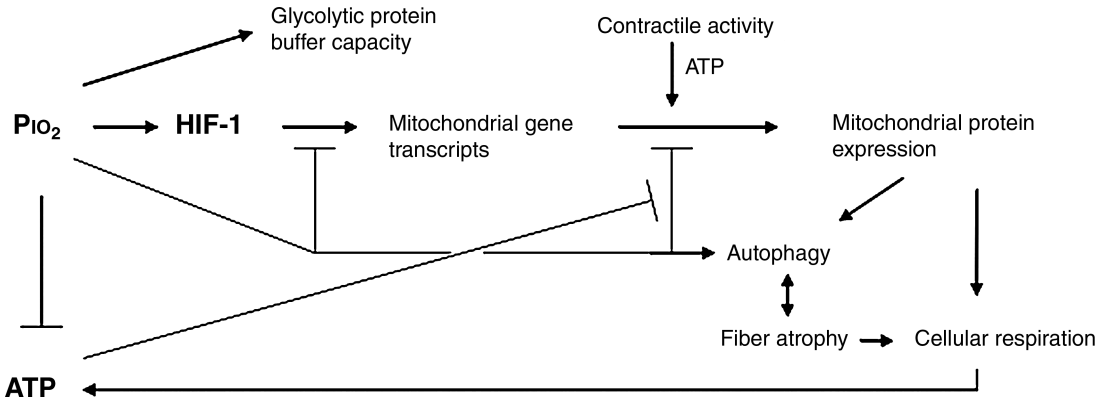

FIG. 4. Sketch summarizing the opposing influence of pulses of muscle deoxygenation for metabolic compensation in recruited muscle by the hypoxia sensor HIF-1 and the noxic consequence of permanently critically low muscle oxygenation for mitochondrial autophagy and related fiber atrophy. The liberated amino acids contribute to the fueling of ATP production. Arrows and blunt-ended lines, respectively, indicate an activating and inhibiting influence.

expected that the role of HIF-1 in muscle signaling is graded by the duration and metabolic intensity of exercise.

Protein measures of HIF-1-regulated genes support the functional relevance of activated HIF-1 signaling in human muscle with muscle work. This is pointed out by investigation into the expression of the HIF-1-dependent vascular endothelial growth factor (VEGF). An upregulation of this factor is believed to contribute to the expansion of the capillary bed in muscle with endurance training (Richardson et al., 1999; Wagner, 2001). Time-course analysis demonstrates an increase in VEGF transcript and protein in human muscle 3 h after intense exercise (Fig. 3). Equally, elevated protein levels of the HIF-1-dependent family of cytochrome c oxidases (Bengtsson et al., 2001; Dapp et al., 2006; Semenza, 2007) after endurance exercise are related to the expressional upregulation of this gene ontology in knee exterior muscle during recovery from exercise (Schmutz et al., 2006). These observations indicate that the threshold for activation of hypoxia-induced mitochondrial and angiogenic gene (and protein) expression is probably slightly above the suggested 3 to 4 torr (Wagner, 2001). Short pulses of hypoxia-induced transcript expression by HIF-1 and the coupling to protein translation evolve as possible mechanisms increasing the levels of aerobic factors with repetition of exercise (Schmutz et al., 2006).

The HIF-1 dependence of gene expression provides an explanation why endurance training under 2500 m of hypoxia does not promote aerobic muscle function (Masuda et al., 2001). This is specifically indicated for the HIF-1-dependent control of the myoglobin gene transcripts which encoded protein product is induced by the intermittent combination of exercise and hypoxia (Terrados et al., 1990; Dapp et al., 2006; Semenza, 2007). This could be equally true for the selective increase of gene messages for the three metabolic factors (glut-4, MCT-1, and CA3) in vastus lateralis muscle. Their upregulation is associated with selective improvements in time-to-exhaustion on a treadmill after interval-type endurance training in hypoxia (Zoll et al., 2006). Protein elevations of these factors contribute to improved glucose uptake and lactate handling and the elimination of carbon dioxide with intermittent hypoxia exposure and training and living at an elevated altitude of 2500 m (Mizuno et al., 1990; McClelland and Brooks, 2002; Chiu et al., 2004). Hypoxia-dependent activation of a gene-dependent program is the probable mechanism by which muscle metabolism is optimized by intermittent work at simulated altitude.

Animal studies imply that the passive lowering of inspired oxygen to a simulate altitude of 5340 m can induce HIF-1α-dependent regulation of mitochondrial, glycolytic, and angiogenic genes in soleus muscle (Dapp et al., 2006; Semenza, 2007). This notion is corroborated by a maintained increase of HIF-1α in rat gastrocnemius muscle after 2 weeks of exposure to hypoxia corresponding to normobaric hypoxia at an altitude of $\sim$5500 m ($F_{IO_2} = 0.10$; De Palma et al., 2007). These observations in rodents are in contrast to the absence of elevations in HIF-1α and mitochondrial protein in vastus lateralis muscle after 5 days of inactive sojourn at 4559 m (Viganò et al., 2008). This does specifically suggest that intramuscular oxygen tension in the studied knee extensor vastus lateralis muscle of subjects is above the threshold for HIF-1α activation. This is possibly explained by a lower degree of contractile activity compared to the highly active postural soleus and gastrocnemius muscles of rodents and the interaction of hypoxia and contractile activity for expression of mitochondrial and capillary factors (Zwetsloot et al., 2008). Thus mitochondrial adaptation in locomotor muscle results from the local oxygen deficit due to the interplay of altitude exposure and contraction-dependent elimination of oxygen in mitochondria.

In this regard, the commonalities in functional adjustments with exercise training in hypoxia to those changes with intermittent hypoxia alone (Pastoris et al., 1994) are noteworthy. They suggest that the repetitive lowering of inspired oxygen fraction with simple breathing induces features known from hypoxia preconditioning of the myocardium (Park et al., 2007). The compartment conferring these improvements of muscle performance remains to be identified (Beidleman et al., 2003).

Conclusions

Real and simulated altitudes induce a catalog of adjustments in muscle proteins and protein assemblies. The observations in selected locomotor muscles support the view that regulation of the muscle proteome by exercise at altitude reflects a trade-off between opposing metabolic strategies to optimize ATP production and energy expenditure (Fig. 4).

Repeated muscle work in moderate heights with concomitant recovery in a well-oxygenated atmosphere elevates the structural components of local aerobic capacity by increased mitochondrial protein expression and modifications toward reduced regulation of ion gradients. Conversely, permanent hypoxia of muscle below a nonidentified threshold causes a net loss of mitochondria, which is compensated for by improved capillary perfusion due to a loss of contractile material.

The lowering of intramuscular oxygen tension below a tentative threshold of 8 torr in combination with nonidentified contraction-related signals is the driving force for elevated angiogenic and mitochondrial protein expression with endurance training. Exercise at moderate altitude exaggerates this response, but protein data are missing to show this for single protein species. Mitochondrial autophagy, fiber atrophy, and possible energy limitations in protein translation prevent the net accumulation of mitochondrial protein after exercise when recovery occurs in an environment above acclimatized altitudes. Conversely, proteins involved in the handling of glucose and lactate at altitude are exempt from muscle wasting (at altitude) and jointly explain, together with improved buffer capacity, the general shift of muscle from aerobic to anaerobic metabolism at altitude. The proteomic evidence supports the tenet of distinctive muscular mechanisms of altitude acclimatization between lowlanders and high-altitude natives. This is highlighted by the selective amelioration of oxidative metabolism in genetically selected highland populations to strenuous exercise at altitude, whereas lowlanders irreversibly suffer from the reduced scope of respiration.

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